Communication

Database on Microbial Thermal Death: Its Construction Design Based on Data from Published Papers and from Experiments Performed under Defined Conditions[†]

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To build a database on the thermal death of microorganisms, we designed a formulated card system consisting of more than 60 items. Those items are displayed on a card on the monitor screen of a personal computer and most of the corresponding parameters for each item are selectable by clicking. Data from research papers published so far and from experiments performed under defined environmental, microbiological, and operational conditions have been collected for the database.

It has often been stressed that so-called predictive microbiology should be introduced for guaranteeing the microbiological safety of foods and studies on this novel approach have recently appeared (Buchanan and Whiting 1996; Fujikawa and Itoh, 1997; Yano, 1998). A substantial amount of data have been collected on the growth and death of various bacteria as influenced by different environmental factors such as temperature, pH, and water activity (e.g., the concentration of sodium chloride). Based upon those data, the database has been built, and further, with the aid of predictive microbiology, two practical computer software programs have been developed for the predictions of food spoilage and preventive effects of a process: "Food Micromodel" in U. K. and "Pathogen Modeling Program" in U. S. A., (McClure et al., 1994; Roberts, 1997; Tsuchido, 1998; Whiting, 1997).

However, the species of bacteria introduced into those database programs are limited to mostly pathogenic ones and little data on spoilage bacteria have been collected yet. Also, the software programs for microbial thermal death, which have been developed so far, do not seem to provide users with sufficiently reliable prediction, compared to those for bacterial growth and spoilage. One of the reasons for such a low reliability in the prediction of thermal death may be the insufficient definition of experimental conditions for the collection of data. In fact, numerous factors have been known to affect the cellular resistance to heat, including factors not only during but also before and after the heat treatment, but also those affecting before and after heat treatment, in contrast to the number of factors involved in microbial growth and spoilage (Tsuchido, 1997). It should be examined which factors are most critical and how those factors affect the heat resistance of a microorganism. Also, it may be desirable that all of the experimental conditions are strictly defined for the determination of heat resistance of a microorganism.

We have previously reported that the thermal history of vegetative cells, such as preincubation

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temperature, postincubation temperature, and rising temperature rate, profoundly affects the heat resistance of cells (Katsui et al., 1981 and 1982; Tsuchido et al., 1974 and 1982). However, for most of researchers, not much attention seems to have been paid to these aspects in the determination of heat resistance of bacterial cells. Therefore, at least part of the often seen substantial differences between the predicted data and observed data on heat resistance may be ascribed to the effects of the thermal history of the cells.

Considering these situations, we started in 1997 to build a thermal death database for microorganisms in two ways. One has been to collect data published so far in some selected journals and the other has been to obtain data from experiments in which various conditions are strictly defined and well controlled.

In the first approach, we have been collecting thermal death data from papers published in several related journals distributed worldwide or journals well-known in Japan, which are listed in Table 1. We are further planning to increase the number of journals to scrutinize. The data from those published papers have been collected from the year 1999 back to the past. Through this approach, it is expected that some suggestive information on reliable predictions of the thermal death of microorganisms could be obtained for practical use.

As for the second approach, we have also been collecting data from experiments performed under defined conditions involving environmental,

microbiological, and operational factors. A strain of *Escherichia coli*, OW6 (Miyake et al., 1993), was chosen as an objective organism and data of its thermal death at different temperatures, different pHs, and different concentrations of sodium chloride have been obtained under strictly defined conditions, which are described in Table 2. To obtain survival data, we have been using the growth-delay analysis method, which has been proposed by Takano and Tsuchido (1982), instead of the conventional colony count method, because of its easiness to use, efficiency and reproducibility of measurement.

The collected data from both approaches have been incorporated into a database system constructed with a database software, FileMaker Pro 4.1 v1 (FileMaker Inc.) using a personal computer (Gateway 2000, P5-66 or Power Macintosh 7600/200). The number of items selected here amounts to as many as 60, including the kind of microorganism, cell form (vegetative cell or spore), conditions of heat activation for spores, the presence of oxygen, redox potential, water activity, kind and concentration of solute dissolved in the heating menstruum, heating apparatus or instrument, D value, Z value, predicted values of $D_{60^\circ\text{C}}$ and $D_{120^\circ\text{C}}$, pattern of survival curve (the appearance of shoulder and/or tailing), method and conditions for the viability assay, in addition to the items of experimental conditions shown in Table 2. All these items are displayed on a monitor screen and most of corresponding parameters for each item can be selected by clicking a pop-up button on the dis-

TABLE 1. List of journals used as sources of data for the building of the database on microbial thermal death as of the year 1999.

Applied and Environmental Microbiology (formerly Applied Microbiology)
Applied Microbiology and Biotechnology (formerly European Journal of Applied Microbiology and Biotechnology)
Biocontrol Science
Bioscience, Biotechnology and Biochemistry (formerly Agricultural and Biological Chemistry)
Biotechnology and Bioengineering
Food Microbiology
Food Science and Technology Research
Food Technology
International Journal of Food Microbiology
Japanese Journal of Food Microbiology (Nippon Shokuhin Biseibutsu Gakkai Zasshi)
Journal of Antifungal and Antibacterial Agents, Japan (Bokin Bobai)
Journal of Applied Microbiology (formerly Journal of Applied Bacteriology)
Journal of Bioscience and Bioengineering (formerly Journal of Fermentation and Bioengineering and Journal of Fermentation Technology)
Journal of Food Hygienic Society of Japan (Shokuhin Eiseigaku Zasshi)
Journal of Food Protection
Journal of Food Science
Journal of Japan Society for Bioscience, Biotechnology and Agrochemistry (Nippon Nogei Kagaku Kaishi)
Journal of Japanese Society of Fisheries Science (Nippon Suisan Gakkaishi)
Journal of Japanese Society for Food Science and Technology (Nippon Shokuhin Kagaku Kogaku Kaishi)
Journal of Society for Bioscience and Bioengineering (Seibutsu Kogaku Kaishi, formerly Hakko Kogaku)
Lebensmittel Wissenschaft und Technologie
Letters in Applied Microbiology

TABLE 2. Basic experimental conditions for obtaining thermal death data of *E. coli*.

Factor	Conditions
Before heat treatment	
For cultivation	
Temperature	37°C
pH	7.0
Growth medium	EM9 broth ^a , 20 ml
Time	16 h (precultivation) and then 2.5 h after inoculation (2% as an inoculum size)
Growth phase	Logarithmic phase (an OD ₆₅₀ of 0.3)
Shaking speed	120 rev/min
For harvesting and washing	
Centrifugation conditions	5,000×g, 5 min, 4°C
Washing	Twice with 50mM potassium phosphate buffer (pH 7.0)
Resuspending conditions	0°C, 3 ml as a final volume
Final cell density	ca. 10 ⁹ cells/ml
For preincubation	
Temperature, time and others	0°C, 60 min, with shaking
During heat treatment	
For starting heat treatment	
Rising temperature process	100-fold dilution method
For isothermal heating	
Temperature	48-56°C
pH	5.5-8.0
Menstruum	50mM potassium phosphate buffer
Initial cell density	10 ⁷ cells/ml
Shaking speed	120 rev/min
Vessel	100-ml flask
Heating apparatus	water-bath incubator
For ending heat treatment	
Falling temperature process	10-fold dilution method; cooled to 37°C, incubated at this temperature for 1 min, and then cooled gradually to 0°C
After heat treatment	
For postincubation	
Temperature and others	0°C, within 1 h, with shaking
For viability measurement by the growth delay method	
Temperature	37°C
pH	7.0
Assay medium	EM9 broth
Inoculation	10%

^a M9 broth supplemented with 0.2% (w/v) glucose and 0.1% (w/v) vitamin-free Casamino Acids (Difco Laboratories).

play. Also, the gram-stain type and optimal growth temperature of bacterium, and the pH of cultivation medium can be automatically recorded from files related to the system.

As described above, the thermal death behavior of the organism is influenced by a variety of factors, some of which are mentioned in this study (Tsuchido, 1997). The set of parameters adopted in this study is only one example among numerous combinations of a variety of factors. In order to build a substantial database which can be available as soon as possible, it is not the best way, or rather impossible, to obtain data for all cases of combinations of experimental conditions. It is useful, therefore, to standardize kinetic formulations expressing dependencies on those

factors and to characterize the thermal death behavior, as one of the possible approaches. The principles of such a standardized method will be described elsewhere.

Once such a database is built up, it should be extremely useful not only for the estimation of the heat resistance of a microorganism under untested novel conditions, but also for the practical predictions regarding the thermal death of target microorganisms, through mathematical modeling, in food pasteurization and sterilization processes. The first results of our on-going work on the construction of a database on microbial thermal death will be published shortly.

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REFERENCES

- Buchanan, R. L., and Whiting, R. C. (1996) Risk assessment and predictive microbiology. *J. Food Prot.*, Suppl., 31-36.
- Fujikawa, H., and Itoh, T. (1997) Present situations and advantages of predictive microbiology (in Japanese). In *Application of Predictive Microbiology to Food* (ed., Yano, N., Kobayashi, T., and Fujikawa, H.), pp. 28-41, Science Forum, Tokyo.
- Katsui, N., Tsuchido, T., Takano, M., and Shibasaki, I. (1981) Effect of preincubation temperature on the heat resistance of *Escherichia coli* having different fatty acid compositions. *J. Gen. Microbiol.*, **122**, 357-361.
- Katsui, N., Tsuchido, T., Takano, M., and Shibasaki, I. (1982) Viability of heat-stressed cells of microorganisms as influenced by preincubation and postincubation temperatures. *J. Appl. Bacteriol.*, **53**, 103-108.
- McClure, P. J., Blackvurn, C. De W., Cole, M. B., Curtis, P. S., Jones, J. E., Legan, J. D., Ogden, I. D., Peck, M. W., Roberts, T. A., Sutherland, J. P., and Walker, S. J. (1994) Modelling the growth, survival and death of microorganisms in foods: the UK Food Micromodel approach. *Int. J. Food Microbiol.*, **23**, 265-275.
- Miyake, T., Araki, S., and Tsuchido, T. (1993) Synthesis and sedimentation of a subset of 15kD heat shock proteins in *Escherichia coli* cells recovering from sublethal heat stress. *Biosci. Biotechnol. Biochem.*, **57**, 578-583.
- Roberts, T. A. (1997) Microbial growth and survival: Developments in predictive modeling. *Food Technol.*, **51**, (4), 88-90.
- Takano, M., and Tsuchido, T. (1982) Availability of growth delay analysis for the evaluation of total injury of stressed bacterial populations. *J. Ferment. Technol.*, **60**, 189-198.
- Tsuchido, T. (1997) Examples of heat-injured microbes and measures against them (in Japanese). In *Technology of Heat Sterilization* (ed., Takano, M., and Tsuchido, T.), pp. 57-68, Science Forum, Tokyo.
- Tsuchido, T. (1998) Building of database and future problems concerning heat sterilization (in Japanese). *Bull. Jpn. Soc. Res. Food Prot.*, **4**, 2-5.
- Tsuchido, T., Takano, M., and Shibasaki, I. (1974) Effect of temperature-elevating process on the subsequent isothermal death of *Escherichia coli* K12. *J. Ferment. Technol.*, **52**, 788-792.
- Tsuchido, T., Hayashi, M., Takano, M., and Shibasaki, I. (1982) Alteration of thermal resistance of microorganisms in a non-isothermal heating process. *J. Antibacterial Antifungal Ag., Jpn.*, **10**, 105-109.
- Whiting, R. C. (1997) Microbial database building: What have we learned? *Food Technol.*, **51**, (4), 82-86.
- Yano, N. (1998) Predictive microbiology and its application in food industry (in Japanese), *Jpn. J. Food Microbiol.*, **15**, 81-87.